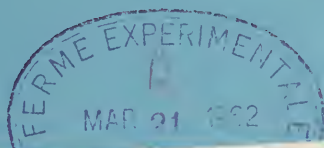


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PUBLICATION 1064

FEBRUARY 1962

CHEMICAL METHODS OF PLANT ANALYSIS



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CANADA DEPARTMENT OF AGRICULTURE



CHEMICAL METHODS OF PLANT ANALYSIS

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RESEARCH BRANCH

CANADA DEPARTMENT OF AGRICULTURE

First Published as Contribution 238

of Chemistry Division, Science Service

Revised — February, 1960

Revised — February, 1962

ROGER DUHAMEL, F.R.S.C.
QUEEN'S PRINTER AND CONTROLLER OF STATIONERY
OTTAWA, 1962

Cat. No. A43-1064

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INTRODUCTION

The Plant Research Institute is responsible for the chemical analysis of a wide variety of plant materials. Both organic and inorganic constituents are determined and the scope of the investigations concerned varies from routine testing to physiological research. A great many samples of horticultural, cereal, and forage plants are analyzed each year for their content of mineral constituents. Many special problems of physiology, growth, nutrition, land management, and plant breeding are investigated.

This publication gives an account of some of the chemical methods of analysis currently in use in the Plant Research Institute. The list of mineral constituents of plants for which methods of determination are given includes all the known nutrient elements and some others not usually considered to be nutrients. Methods are also given for a few of the more commonly determined organic constituents for which standard procedures have been developed.

The science of analytical chemistry is constantly producing new and improved techniques. The methods presented here are not the only ones available. They have, however, been selected and in some cases adapted because they represent the best known procedures in respect to accuracy, speed, and ease of manipulation. Some of the methods presented have been in use for many years and have proved to be very reliable. Other methods have been only recently adopted. Since the appearance of the first edition of this manual in 1953, several new methods have been put into use and the procedures are included in this edition.

The authors wish to acknowledge the assistance of several colleagues in the preparation and testing of the methods presented here. Special thanks are due to J.R.W. Miles, A.B. Durkee, J.C. Sirois, Miss M.M. Cote, and Miss A. G. Savage.

Following the outline of each method a list of references is presented which constitutes an acknowledgment of material used in this publication. References are also included which give details of procedure for alternative methods of analysis for some constituents. The following references are suggested as sources of general information for those engaged in plant chemistry:

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PREPARATION OF SAMPLES

The procedure to be used in the preparation of samples of plant material for analysis depends to a certain extent upon the purpose of the analysis and upon the nature of the constituents that are to be determined. Most living plant tissue contains a large proportion of water. Some analytical determinations are made directly upon fresh material while others are made upon dried tissue only. Dried tissue can be safely and conveniently stored and for this reason is frequently used for analysis. However as the drying process may produce undesirable changes in some chemical constituents of the sample, certain analyses must be performed immediately on fresh tissue.

The preparation of plant material for analysis is just as important as the analytical procedure and should be carried out with the same careful attention to detail. A procedure for preparation is outlined below.

Segregation of Tissues

It is seldom useful or necessary to mix all parts of a plant into a composite sample for analysis. Such a practice may be necessary for the determination of the total nutrient value of a feed or forage but an adequate interpretation of plant growth phenomena can be made only by examining the levels of constituents in individual tissues or organs. Sharp knives or dissecting scissors are the most useful instruments for making this separation or segregation. The degree of separation is often limited by considerations of practicability when many samples are involved, but as a general rule it is better to discard part of the plant rather than make a composite sample of organs of dissimilar structure and composition. Corresponding plant parts from more than one plant may be composited to obtain representative sampling or samples of sufficient size for analysis.

Cleaning of Tissues

Plant tissues, particularly from the lower portions of field-grown plants, are often contaminated with soil or other material. Before analysis this must be removed as completely as possible. During the operation of harvesting, plants intended for analysis should not be laid on the ground. The gummy exudations on the surfaces of many types of plants will pick up particles of soil which are difficult to remove. It is much simpler to avoid contamination than to remove it or correct for it. It is often possible to avoid soil contamination by refraining from collecting field samples immediately after a rain or heavy dew, and consequently allowing the surface of the plants to dry before harvesting. Washing plant tissues with water is not recommended as a general method of cleaning, although roots will occasionally have to be washed free of soil or sand. Gentle brushing with a stiff-

bristled brush is usually effective in removing most contamination. Procedures to be followed with samples not properly cleaned before analysis are described in subsequent sections.

Subdividing Green Tissue

When the tissues have been separated into individual samples and cleaned, each sample should be immediately cut into small pieces with a sharp knife or scissors and thoroughly mixed. Portions of this sample may then be weighed out for those analyses that are performed directly upon green material or for the determination of the total moisture content of the green tissue. All operations in the preparation of a sample up to this point must be carried out as rapidly as possible and with due precaution to prevent loss of moisture from the harvested plants. Whole plants or leaves may be placed in a container with the bases of stalks or stems in water or they may be covered with a rubber or plastic sheet to prevent excessive wilting. When analyses are to be made upon dried material only and reported as a fraction of the total dry weight, it is unnecessary to observe the above precautions.

Expressed plant sap or juice is sometimes required for analysis. In such instances the plant sample is prepared as outlined above and a definite quantity is weighed and passed through a juice extractor or subjected to pressure in a press. When the latter procedure is employed, the sample is first placed in a cheesecloth bag.

Drying Green Tissue

The cleaned, cut tissue may be dried for storage. Satisfactory results may be obtained by any one of several methods. Best results are probably obtained by placing samples in cheesecloth bags in an oven with a forced draft at a temperature 70°C. Drying may also be done in a vacuum oven at 70°C. or in a standard oven at 100°C. Green tissue should not be tightly packed in a container for drying, otherwise scalding will occur. Scorching by overheating should also be avoided.

For the determination of the total moisture content of green tissue, weigh a 50-gram sample into a large shallow container and heat in a vacuum oven at 70°C. or in a standard oven at 100°C. The duration of time for heating will vary with the type of tissue; 18 hours is sufficient for most tissues. After heating, cool the sample in a desiccator and reweigh. The sample may be reheated until there is no further loss in weight. The total loss in weight is calculated as per cent moisture. Some tissues which contain volatile organic constituents or high percentages of sugars cannot be brought to a constant weight and in such cases a compromise procedure must be adopted. A standard technique and period of heating should be adopted for each type of tissue being analyzed. If only a small quantity of green tissue is available for analysis, a sample smaller than 50 g. may be taken for the determination of the moisture content. After the determination of the moisture content the dry residue may be used for further analysis if necessary.

Because loss of moisture through wilting commences as soon as the plant is cut, the determination of the moisture content of green tissue should be carried out as soon as possible after a plant has been harvested.

Grinding Dry Tissue

Dry tissue is commonly ground to a fine powder before analysis to ensure representative sampling and complete extraction in analyses involving solvent extraction procedures. Mechanical mills are most satisfactory for this purpose. It is extremely important that the mill be thoroughly cleaned between the grinding of individual samples. A stiff-bristled brush is effective for this purpose and an air blast or a vacuum suction is a useful aid. Grinding with a mortar and pestle is necessary in special instances, such as in analysis for molybdenum. Samples should be ground to pass a 60-mesh sieve for most analyses. All of the sample must be ground and screened and then thoroughly mixed. Ground samples should be stored in clean bottles or polyethylene vials fitted with tight covers and should be clearly and correctly labeled. Samples prepared in this way can be stored indefinitely.

PREPARATION OF EXTRACTS

Before any chemical analysis of plant tissue can be performed it is necessary to prepare a solution or extract which contains the constituent that is to be measured. In addition to putting the constituent into a measurable form, this procedure eliminates many interfering substances. Some extracts are prepared to contain the total quantity of a given constituent found in the plant tissue sample. Others contain only specific fractions of the constituent which are separated by means of selective extraction. When either the required fraction or the total quantity of a constituent has been extracted, the same chemical method of analysis can usually be applied to either extract.

Total amounts of mineral constituents in plant tissue are usually determined by ashing a sample and dissolving the ash in dilute acid. For some determinations a compound is added to the sample before ashing in order to aid in combustion or to prevent volatilization. Many variations of the ashing procedure have been advocated, particularly in reference to duration and temperature of ashing. Certain materials require more drastic treatment than others to render them easily soluble for analysis. One standard procedure is given below. Other procedures may give equally reliable results.

In plant nutrition it is often necessary to get a lot of chemical analyses completed in a short time, but absolute accuracy is not essential. For this purpose 'rapid tests' have been developed, some of which are quite empirical. Usually these tests are made on extracts of fresh green tissue made with water or very weak acid. One method of preparing an extract of soluble mineral constituents is given below. No methods are given in this publication for rapid field tests, but several laboratory procedures are described that can be called rapid methods. It must be emphasized that these methods give results that are not so accurate as those obtained by other standard procedures.

Method No. 1 for potassium, Method No. 1 for calcium, and Method No. 1 for magnesium give results that are not consistently within the 5 per cent limit of error generally allowed for routine analysis of plant tissues. The method for total soluble nitrogen, though a rapid one, gives accurate results.

Organic solvents are commonly used for preparing extracts of organic constituents. Methods for preparing extracts of sugars and other organic constituents are given below.

Ash Extract of Total Mineral Constituents

Weigh 1.000 g. of dried tissue into a silica dish. Place in a muffle furnace and raise the temperature gradually to 550°C. Continue heating for 2 hours. Cool, add 20 ml. water and 1 ml. HCl and warm on a hot plate for a few minutes. Filter into a 100-ml. volumetric flask. Wash residue thoroughly with water and allow the filter paper to dry slightly. Place filter paper with residue in the original silica dish, return to the muffle furnace and ignite at a temperature of 550°C. for about 30 minutes or until a white ash is obtained. Cool, add 5 ml. water and 3 drops HCl. Heat to boiling on a hot plate, cool and filter into the original volumetric flask. Wash residue thoroughly with water.

This residue may be transferred to a tared porcelain crucible and ignited at 550°C. for 1 hour. The weight of ignited precipitate may be reported as percentage of insoluble residue. This component represents sand on the surface of the original tissue and a small amount of plant silica. If the percentage is large, a corresponding correction must be made in calculating the amounts of all other constituents for which the sample is analyzed. In such cases results of analysis for iron, manganese, etc., will be unreliable depending on the composition of the contaminant.

The filtrate in the 100-ml. volumetric flask is made up to volume, and appropriate aliquots are used in the determination of the following constituents: phosphorus, potassium, sodium, calcium, magnesium, iron, manganese, copper. In the application of some of the methods described in the following pages to certain types of plant tissue it may be necessary to adjust the concentration of this extract by dilution or by ashing a smaller or larger weight of sample.

Special procedures are required in the preparation of extracts for the determination of total chlorine, sulphur, boron, zinc, molybdenum, and cobalt. These are described in detail in the procedures for the respective methods.

In the preparation of standard solutions in a number of the colorimetric procedures described in the following pages a quantity of acid equivalent to that in the extract being analyzed must be added to the standard. Acid solutions of these concentrations are referred to in the various procedures as 'extracting solutions' or 'the required amount of acid'. The extracting solution will vary in type and strength according to the extract being analyzed.

When inorganic analyses are to be made on plant samples that are grossly contaminated with soil, a fairly complete separation may be obtained with the following procedure:

Weigh accurately a quantity of dried, ground tissue into a 250-ml. beaker. Add chloroform or carbon tetrachloride and mix thoroughly. Allow to

settle for a few minutes and then decant. Stir decanted mixture, allow it to settle and decant it again. Repeat the procedure until no further soil settles to the bottom. Using fresh liquid, repeat the procedure with the separated portions of soil. Collect all liquid and all plant material in a silica dish, evaporate to dryness, and follow the ashing procedure outlined above. Collect the residue of soil, ignite and weigh in the manner described above for 'insoluble residue'. In the calculation of results an appropriate correction must be applied.

Extract of Soluble Mineral Constituents

Weigh 5 g. of finely cut green tissue into a Waring blender. Add about 1 g. of activated carbon and 50 ml. of 2 % (v./v.) acetic acid. Allow blender to run for 5 minutes. Filter immediately but do not wash the precipitate. Store the extract in a closed container in a refrigerator until the analyses are to be made.

Extract of Soluble Sugars

Weigh 5 g. of finely cut green tissue into a 300-ml. extraction flask. Add 200 ml. 80 % ethyl alcohol and boil on a hot plate under a reflux condenser for 4 hours. To ensure complete extraction with certain types of tissue decant off the alcohol, add 200 ml. of fresh alcohol and continue the extraction for 2 hours. OR Weigh 5.000 g. of dried ground tissue into an extraction thimble. Place thimble in a Soxhlet extractor and extract with 80 % ethyl alcohol for 8 hours. For the extraction of fruits or other highly acidic tissues it is recommended that a small quantity of CaCO_3 be added to the extracting solution to prevent hydrolysis of oligosaccharides.

Upon completion of either type of extraction, filter the alcohol into a Claissen flask, wash the tissue with fresh alcohol and dry the residue for the determination of starch. Distil the alcohol extract under vacuum in a water bath at 40°C . until a very small volume remains. Transfer this residue quantitatively to a 50-ml. centrifuge tube, using glass beads and a vigorous swirling motion to clean the flask with successive 5-ml. portions of water. Add the wash water to the residue in the centrifuge tube. Add 1 ml. saturated neutral lead acetate, mix and centrifuge at 1000 $\times$ g. for 5 minutes. Test the supernatant liquid with one drop of lead acetate for completeness of precipitation, then transfer the liquid to another 50-ml. centrifuge tube. Wash the precipitate with 5 ml. of water, centrifuge for 5 minutes and add the wash water to the original supernatant. Add just enough solid Na_2CO_3 to precipitate the excess lead, mix and centrifuge for 5 minutes. Again test the supernatant for completeness of precipitation with a few granules of Na_2CO_3 . Transfer the liquid to a 50-ml. volumetric flask. Wash the precipitate with 5 ml. of water, centrifuge for 5 minutes and add the wash water to the sample in the flask. Make to volume with water. Sugars may be determined on this solution by any of a number of procedures. Adjustment of concentration may be necessary with certain extracts before analysis can be made.

CALCULATION OF RESULTS

There are several methods for expressing the results of a quantitative analysis of plant tissue. The weight of an individual constituent may be calculated as a proportion of the total dry weight or of the total green weight of the tissue. It may be calculated to a standard moisture content or it may be expressed on the biometric basis, that is, total weight per unit of growth. The unit selected may be one or more whole plants or one or more whole organs. Mineral constituents may be expressed as a proportion of the total ash of a sample. The method of expression is sometimes chosen for reasons of convenience in reporting but it is also true that certain plant-growth phenomena and physiological relationships that are not apparent from one method of expression are quite obvious when analytical results are expressed on another basis.

It is customary to report the total amounts of the major nutrient elements as percentages of dry weight, sometimes as percentages of ash. Micro-nutrient elements are expressed as parts per million of dry weight. The soluble fractions of the mineral elements are expressed as parts per million of green weight. Organic constituents are usually expressed as either percentage of dry weight or percentage of green weight. Any constituent may be expressed on the biometric basis for a particular reason. All mineral constituents are calculated and expressed as the element and not as the oxide.

If desired, the calculation of results may be facilitated by adjusting sample weights, when weighing out samples for analysis, so that they contain a weight of dry matter in whole numbers of grams. The percentage of moisture in the sample is employed to determine the weight of sample to be taken, as in the following example:

Moisture found	5%
Recommended sample weight	2 g.
Sample weight used	$\frac{2.00}{.95} = 2.105 \text{ g.}$

When a plant sample is grossly contaminated with soil or sand that cannot be removed, a determination of sand or insoluble residue should be made as directed above and the results of subsequent analyses should be calculated to a 'sand-free' as well as a 'moisture-free' basis to obtain the best approximation possible under the circumstances.

EQUIPMENT AND CHEMICALS

In addition to the usual standard laboratory glassware and apparatus a few pieces of special equipment are required in the methods of analysis outlined in the following pages. A large muffle furnace with adequate temperature control and pyrometer is essential. A centrifuge that can accommodate both 50-ml. and 15-ml. centrifuge tubes is recommended. The method outlined for the determination of zinc requires a polarograph. One of the methods for starch makes use of a polarimeter. A constant temperature water bath is required in several of the procedures.

Some type of laboratory oven is necessary as well as a sensitive analytical balance. Silica dishes of convenient size are recommended for most ignitions. Platinum dishes are sometimes required. The Waring blender has become a standard item of equipment for making certain types of extractions. Soxhlet extractors are used for other extractions. The determination of ether extract requires some type of standard equipment designed for this purpose, such as the Goldfish extraction unit.

Most of the methods outlined are colorimetric procedures and a photoelectric colorimeter is required to carry out these determinations. All the methods have been tested on a Klett-Summerson photoelectric colorimeter, and this instrument has been found to be sufficiently sturdy and accurate for purposes of routine analysis. Other photoelectric colorimeters will undoubtedly give equally reliable results. The numbers for filters recommended refer to those that are used with the Klett-Summerson instrument. A spectrophotometer may, of course, be substituted for the colorimeter in colorimetric methods. Indeed, it is recommended in the method for cobalt. A flame photometer is required for two new methods included in this edition: those for determination of potassium and sodium.

It is recommended that a set of six closely matched colorimeter tubes be used in the colorimetric work, particularly in the preparation of standard curves. When tested with water or the test solution on the colorimeter, the tubes in any one set should not show a difference of more than one division on the instrument scale.

Mixing of solutions in the colorimeter tubes is done by means of a bubbler tube, which is simply a straight piece of glass tubing about ten inches in length that has been drawn out to a long capillary tip at one end. The fine tip is inserted in the solution and the operator blows gently. Washing or rinsing the tip between samples is unnecessary. In the procedure for determining nitrogen a rubber bulb may be used for blowing through the bubbler to avoid possible ammonia contamination from the breath.

In the procedures outlined in the following pages all reagents mentioned are of Reagent grade or corresponding quality. The commonly used reagents such as the mineral acids, ammonia, etc., are not included in the lists of reagents for individual methods. Where they are mentioned in procedures it is understood that the Reagent grade concentrated reagent is intended to be used. Where water is mentioned in any procedure, this is understood to be distilled water. Concentrations of reagent solutions are expressed as percentage values. In those instances where a solid reagent is to be dissolved in water and diluted to a particular concentration the designation (w/v) is understood but not printed.

Example : 20 % means that 20 g. of solid reagent is to be dissolved in water and the resulting solution diluted to a total volume of 100 ml. It does not mean that 20 g. of solid reagent is to be dissolved in 100 ml. of water. Concentrations of liquid reagents are also indicated as percentage values, but the designation (v/v) is used to indicate that a given volume of concentrated reagent is to be diluted to a total volume of 100 ml. with water unless other instructions are given.

MOISTURE

Weigh 2.000 g. of air-dried, ground tissue into a tared porcelain crucible. Dry in a vacuum oven at 70°C. or in a standard oven at 100°C. for 18 hours. Place in a desiccator for 15 minutes and reweigh. Desiccators should be charged periodically with fresh calcium chloride. The loss in weight is calculated and reported as per cent moisture.

TOTAL ASH

After determination of moisture place the crucible and contents in a muffle furnace and raise the temperature to 550°C. Continue heating at this temperature for 2 hours. Remove crucible to a desiccator, cool for 30-45 minutes, and reweigh. The weight of the residue in the crucible is calculated and reported as per cent ash. If black specks of unburned carbon persist in the ash after 2 hours of heating, it may be necessary to prolong the time of combustion. The process may be accelerated by cautiously stirring up the ash with a fine platinum wire.

NITROGEN

Principle

Turbidimetric method. Compounds of nitrogen are converted to ammonia which forms a brownish compound with the mercuric potassium iodide of Nessler's solution. The brownish compound is measured turbidimetrically.

Reagents

1. Digestion reagent

Sulphuric acid	--	H_2SO_4	75 ml.
Phosphoric acid	--	H_3PO_4	25 ml.
Copper sulphate	--	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	1 g.
Selenous acid	--	H_2SeO_3	1.2 g.

Grind the two solids separately and dissolve each in one half the phosphoric acid. Mix the two acid portions and add the sulphuric acid.

2. Rochelle salt solution

Dissolve 50 g. of potassium sodium tartrate, $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$, in 100 ml. water.

3. Nessler's reagent

Stock solution: Weigh 150 g. potassium iodide, KI, and 110 g. iodine, I_2 , into a 500-ml. florence flask. Add 100 ml. water and an excess of metallic mercury (140-150 g.). Shake the flask continuously and vigorously. The solution becomes quite hot. When the red iodine solution has just begun to become noticeably lighter in color, though still red, cool in running water and continue the shaking until the reddish color of the iodine has been replaced by the greenish color of the double iodide.

The whole operation usually takes about 15 minutes. Separate the solution from the surplus mercury by decantation. Wash the mercury with liberal quantities of distilled water. Dilute the solution and washings to 2 liters. If the cooling is begun soon enough, the resulting reagent is clear enough for immediate dilution with 10% alkali and may be used at once.

Reagent: Measure into a large bottle 3500 ml. 10% NaOH prepared by diluting a clear saturated solution. Add 750 ml. mercuric potassium iodide and 750 ml. water.

4. Sodium hydroxide -- NaOH, 8%

5. Titanous chloride -- TiCl_3 , 12.5%

Caution: Do not shake bottle or disturb precipitate on the bottom.

6. Ammonium sulphate -- $(\text{NH}_4)_2\text{SO}_4$

Procedure for Total Nitrogen

Digestion: Weigh 0.100 g. dried material into a 100-ml. Kjeldahl flask. Add 2 ml. digestion reagent (Reagent 1) and two glass beads or teflon boiling chips. Heat gently over a flame until foaming commences, then withdraw the flame until foaming subsides. Heat strongly until condensing vapors bring down any spattered material from the walls of the flask. Continue boiling until mixture changes from a chocolate brown to a clear pale green, usually about 10 to 12 minutes; then boil for an additional 2 to 3 minutes. If the liquid stops boiling during the heating period, superheating may occur followed by a violent eruption and loss of the sample. To prevent this, give the flask a sharp tap with a small mallet made from a rubber stopper and a stirring rod. Cool, dilute to 250 ml. with water.

As an alternative procedure the digestion may be carried out using a cylindrical aluminum block set upon a heavy-duty 2000-watt hot plate equipped with a three-heat switch. Digestions should be made in special thick-walled resistance-glass rimmed test tubes 25x150 mm. of approximately 30-ml. capacity. The tubes fit loosely into one-inch holes bored to a depth of two inches in the block. Measure 0.100 g. dried sample and 2 ml. digestion reagent (Reagent 1) into a test tube, add a teflon boiling chip, heat the block to 370°C., lower the test tube into the block and continue the heating for the time necessary to complete the digestion as described above.

Determination: Measure 1 ml. of digested solution into a colorimeter tube. Add 0.5 ml. Rochelle salt solution (Reagent 2). Make volume to 6 ml. with water and mix. Add 1 ml. Nessler's solution (Reagent 3) and mix. Read absorbance immediately in a photoelectric colorimeter using a No. 42 blue filter. Set the instrument scale at zero with water.

Procedure for Total Soluble Nitrogen

Measure 5 ml. of extract into an Erlenmeyer flask. Add 20 ml. water, 6 ml. sodium hydroxide (Reagent 4) and mix thoroughly by swirling. Add 1 ml. titanous chloride (Reagent 5) and mix again. (Mixing in both instances should be thorough but not violent or prolonged.) Allow to stand 5 minutes. Make up to 40 ml. with water. Filter. Immediately measure 30 ml. of filtrate into a 125-ml. Kjeldahl flask, add 2.5 ml. digestion reagent (Reagent 1) and heat as directed above in Procedure for Total Nitrogen.

The digestion may be carried out in a test tube in the aluminum block as described above, but the aliquot together with the digestion reagent must first be reduced to a volume of about 5 ml. by heating in an oven at 105°C. for several hours. Dilute the final digest to a volume of 100 ml. before Nesslerization.

Standard

Dissolve 0.4717 g. ammonium sulphate (Reagent 6) in water and make to 1 liter. Dilute 10 ml. of this solution to 100 ml. with water.
1 ml. = 10 micrograms N.

To prepare a standard curve use aliquots of the standard solution from 0.1 ml. to 1.0 ml., making each aliquot to a total volume of 1.0 ml. with water.

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NITRATE NITROGEN

Principle

Colorimetric method. Nitrates are dissolved in an aqueous solution from dried plant tissue; chlorides and organic matter are eliminated. In alkaline solution a yellow compound is formed with a phenol sulphonic acid reagent and is measured colorimetrically.

Reagents

1. Copper sulphate-- $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.5% solution
2. Silver sulphate-- Ag_2SO_4 , 0.35% solution
3. Sodium phosphate-- NaH_2PO_4

Dissolve 138 g. in 500 ml. water, add strong NaOH solution to bring pH to 6.5 and make to 1 liter.

4. Calcium hydroxide - magnesium carbonate mixture-- $\text{Ca}(\text{OH})_2$, MgCO_3
Triturate 1 part of $\text{Ca}(\text{OH})_2$ and 2 parts of MgCO_3 in a mortar.

5. Phenol-p-sulphonic acid

Leave H_2SO_4 in contact with 1 drop of mercury overnight to free it from nitric acid. Add 25 g. phenol, $\text{C}_6\text{H}_5\text{OH}$, to 225 ml. H_2SO_4 . Heat on steam bath for 2 hours.

6. Ammonium hydroxide-- NH_4OH , 50% (v/v)
7. Potassium nitrate-- KNO_3 , 0.0505% solution.
1 ml. = 0.07 mg. N

Procedure

Weigh 0.100 g. dried tissue into a small beaker or flask. Add 9 ml. silver sulphate (Reagent 2) and swirl quickly. Add immediately 1 ml. sodium phosphate (Reagent 3). Allow to stand 2 hours. Filter. Measure 1 or 2 ml. of filtrate into a 15 ml. centrifuge tube, add 2 ml. copper sulphate solution (Reagent 1), mix, add water to make 6 ml. and approximately 0.5 g. Reagent 4. Mix and allow to stand 1 hour, centrifuge.

Measure 2 ml. phenol sulphonic acid (Reagent 5) into a boiling tube, directly onto the bottom. Add by drops 2 ml. supernatant from above directly onto the reagent, swirling carefully after the addition of each drop. Cool, and add cautiously with stirring 25 ml. ammonium hydroxide (Reagent 6). Cool, and read absorbance in a photoelectric colorimeter using a No. 42 blue filter and with the instrument set at zero with water.

Standard

Use aliquots of standard solution (Reagent 7) from 1 to 4 ml. and follow the above procedure beginning with the addition of copper sulphate.

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POTASSIUM

Method No. 1

Principle

Turbidimetric method. A finely divided yellow precipitate of sodium potassium cobaltinitrite is formed at a controlled temperature and pH. It remains in suspension and is measured turbidimetrically.

Reagents

1. Sodium cobaltinitrite solution
 - (a) Dissolve 25 g. cobalt nitrate, $\text{Co}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$, in 50 ml. water. Add 16 ml. acetic acid.
 - (b) Dissolve 90 g. sodium nitrite, NaNO_2 , in 150 ml. water. Add (b) to (a) slowly, aerate for 6 hours and filter. Aerate for a few minutes each day before using. Store in refrigerator and prepare fresh monthly.
2. Sodium acetate solution
Dissolve 2 g. sodium acetate, $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, in 100 ml. ethyl alcohol. Grind the sodium acetate and warm the alcohol to facilitate solution.
3. Alcoholic sodium cobaltinitrite
Warm Reagent 2 to 28°C . in a water bath. Mix 10 ml. of the warmed Reagent 2 and 0.5 ml. of Reagent 1. Prepare this mixture fresh immediately before use and shake before the removal of each aliquot. Deliver the required aliquot with a fast-flowing pipette.
4. Sodium acetate-- $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, 40 % solution
5. Potassium chloride-- KCl

Procedure

Measure 3 ml. extract into a colorimeter tube. Add 2 ml. extracting solution, 6 drops sodium acetate buffer (Reagent 4) and mix. Place tube in a constant temperature water bath at 28°C . and allow to stand 5 minutes. Add 5 ml. alcoholic sodium cobaltinitrite (Reagent 3), allowing reagent to flow down the side of the tube to form a layer on the top. Shake the tube at once vigorously several times. Read the absorbance immediately in a photoelectric colorimeter with a No. 66 red filter. Set the instrument scale at zero with water.

Standard

Dissolve 0.9534 g. potassium chloride (Reagent 5) in water, add the required amount of acid and make to 1 liter.

1 ml. = 0.5 mg. K.

To prepare a standard curve use aliquots of the standard solution from 0.2 ml. to 1.0 ml., making each aliquot to a total of 5.0 ml. with extracting solution.

References

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POTASSIUM

Method No. 2

Principle

Volumetric method. In acid solution potassium is precipitated as the yellow double cobaltinitrite salt. The salt is dissolved in hot dilute acid and titrated with standard potassium permanganate. Since the end point is indeterminate, a standard quantity of sodium oxalate is added at the end of the titration to produce a sharp end point.

Reagents

1. Sodium acetate-- $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, 40 % solution
2. Sodium cobaltinitrite solution-- $\text{Na}_3\text{Co}(\text{NO}_2)_6$:
 - (a) Dissolve 25 g. cobalt nitrate, $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, in 50 ml. water and add 12.5 ml. glacial acetic acid.
 - (b) Dissolve 140 g. sodium nitrite, NaNO_2 , in 210 ml. water. Slowly add (b) to (a). Bubble air through the solution for 3 hours. Filter and store in refrigerator. Aerate solution for fifteen minutes and filter if necessary before using.
3. Sulphuric acid-- H_2SO_4 , 12 % (v/v)
Add acid to water slowly, stirring constantly; cool, and make up to volume.
4. Acetone-water mixture-- 300 ml. water + 100 ml. acetone, CH_3COCH_3
5. Dry acetone
Add anhydrous sodium carbonate, Na_2CO_3 , to acetone CH_3COCH_3 in the proportion of 10 g. per liter and store in this condition.
6. Sodium oxalate solution--0.01 N $\text{Na}_2\text{C}_2\text{O}_4$ (0.067 %)
Before preparing this solution heat the sodium oxalate overnight at 105°C . and cool in a desiccator.
7. Potassium permanganate-- KMnO_4 ,
Stock solution 0.1 N: Dissolve 3.16 g. in water and make to a volume of 1 liter. Store in a dark-colored bottle.
Standard solution 0.01 N: Dilute 10 ml. of stock solution to 100 ml. with water. Prepare fresh before using. Standardize by titrating against sodium oxalate solution (Reagent 6) acidified with 15 ml. dilute sulphuric acid (Reagent 3) Heat to 80°C . before titrating.

$$\text{Normality of } \text{KMnO}_4 = \frac{\text{g. } \text{Na}_2\text{C}_2\text{O}_4}{\text{ml. } \text{KMnO}_4 \times 0.067} \quad \text{or} \quad \frac{\text{ml. } \text{Na}_2\text{C}_2\text{O}_4 \times \text{normality of } \text{Na}_2\text{C}_2\text{O}_4}{\text{ml. } \text{KMnO}_4}$$

1 ml. 0.01 N KMnO_4 = 0.07 mg. K approx. (May vary slightly with conditions of experiment. Factor should be determined with standard potassium solutions).

Procedure

Measure 1 ml. of extract into a 15-ml. centrifuge tube. Add 3 ml. water, 1 ml. sodium acetate solution (Reagent 1) and 1 ml. sodium cobaltinitrite solution (Reagent 2), the last being added by drops. Mix and allow to stand for 2 hours at 5°C. Centrifuge at 1000 $\times$ g. for 15 minutes and decant the supernatant liquid. Add 5 ml. acetone-water mixture (Reagent 4), mix, centrifuge for 15 minutes, and decant the wash solution. Repeat the washing procedure using acetone (Reagent 5). Allow the precipitate to stand a few minutes for the acetone to evaporate. Add a little standard potassium permanganate (Reagent 7) from a burette, then add 2 ml. dilute sulphuric acid (Reagent 3). Complete the reaction by adding permanganate from the burette with the tube set in a container of boiling water and with constant shaking, always maintaining an excess of permanganate. When the precipitate is completely dissolved and a permanent pink color is obtained, add 2 ml. sodium oxalate solution (Reagent 6) and titrate to an end point.

True titration value = ml. 0.01 N KMnO_4 solution
minus ml. 0.01 N $\text{Na}_2\text{C}_2\text{O}_4$ solution.

References

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- McCance, R.A., and H.L. Shipp. The chemistry of flesh foods and their losses on cooking. Med. Res. Council. Spec. Rept. Series No. 187. 1933.
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POTASSIUM

Method No. 3

Principle

Flame photometric method. Potassium in solution is atomized into an oxyhydrogen or oxyacetylene flame. The flame excites atoms of potassium causing them to emit radiations at specific wave lengths. The amount of radiation emitted is measured on a spectrophotometer. Under standard conditions it is proportional to the concentration of potassium in solution.

Reagents

1. Potassium chloride-- KCl, 0.572% solution.

Stock standard solution contains 3000 p. p. m. K.

2. Standard solution

Measure 50 ml. stock standard solution (Reagent 1) and 5.0 ml. HCl into a flask and make solution to a volume of 1 liter. Standard solution contains 150 p. p. m. K. To prepare a standard curve use aliquots of this solution from 0 to 150 ml. making each aliquot to a volume of 150 ml. with 0.5% HCl (v/v). Atomize as described below, setting the top standard at 100% transmittance.

In order to compensate for minute interferences produced by other ions in the flame photometric determination of potassium, it is recommended that the standard solution be augmented with approximately equivalent concentrations of those ions that occur in highest proportions in the tissue being analyzed. A background of emission spectra roughly similar to what might be found in an average plant extract is obtained when the standard contains 150 p. p. m. calcium, 75 p. p. m. magnesium, 15 p. p. m. phosphorus.

Procedure

Dilute an aliquot of plant extract so that it contains less than 150 p. p. m. K. Add sufficient HCl so that the concentration of acid is the same as that in the standard solution. Atomize the diluted extract in a calibrated flame photometer with the wavelength dial set at 768 millimicrons and the transmittance set at 100% for the top standard solution of potassium. Check the instrument periodically with the top standard solution.

SODIUM

Principle

Flame photometric method. Sodium in solution is atomized into an oxyhydrogen or oxyacetylene flame. The flame excites atoms of sodium causing them to emit radiations at specific wave lengths. The amount of radiation emitted is measured on a spectrophotometer. Under standard conditions it is proportional to the concentration of sodium in solution.

Reagents

1. Sodium dihydrogen phosphate - $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.12 % solution.

Stock standard solution contains 200 p. p. m. Na.

2. Standard solution

Measure 50 ml. stock standard solution (Reagent 1) and 5.0 ml. HCl into a 1-liter volumetric flask and make to volume with water. Standard solution contains 10 p. p. m. Na. To prepare a standard curve use aliquots of this solution from 0 to 150 ml. making each aliquot to a volume of 150 ml. with 0.5 % HCl (v/v). Atomize as described below, setting the top standard at 100 % transmittance.

In order to compensate for minute interferences produced by other ions in the flame photometric determination of sodium, it is recommended that the standard solution be augmented with approximately equivalent concentrations of those ions that occur in highest proportions in the tissue being analyzed. A background of emission spectra roughly similar to what might be found in an average plant extract is obtained when the standard contains 150 p. p. m. potassium 150 p. p. m. calcium, 75 p. p. m. magnesium, 15 p. p. m. phosphorus.

Procedure

Dilute an aliquot of plant extract so that it contains less than 10 p. p. m. Na. Add sufficient HCl so that the concentration of acid is the same as that in the standard solution. Atomize the diluted extract in a calibrated flame photometer with the wavelength dial set at 589 millimicrons and the transmittance set at 100 % for the top standard solution of sodium. Check the instrument periodically with the top standard solution.

PHOSPHORUS

Method No. 1

Principle

Colorimetric method. A phosphomolybdate complex is first formed and then reduced with amino-naphthol-sulphonic acid to the complex molybdenum blue which is measured colorimetrically.

Reagents

1. Perchloric acid-- HClO_4 , 72 %
2. Ammonium molybdate-- $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 5 % solution
3. Amino-naphthol-sulphonic acid solution
Dissolve in water 0.5 g. 1-amino-2-naphthol-4-sulphonic acid, 30 g. sodium bisulphite, NaHSO_3 , and 6 g. sodium sulphite, Na_2SO_3 . Make to 250 ml. Allow to stand overnight and filter. Prepare fresh every two weeks.
4. Potassium dihydrogen phosphate-- KH_2PO_4

Procedure

Measure 2 ml. of extract into a colorimeter tube. Add 4 ml. water 0.5 ml. perchloric acid (Reagent 1) and mix. Add 0.5 ml. ammonium molybdate solution (Reagent 2) and mix. Add 0.25 ml. amino-naphthol-sulphonic acid solution (Reagent 3), and mix. Allow to stand 10 minutes. Read absorbance in a photo-electric colorimeter using a No. 66 red filter. Set the instrument scale at zero with water.

Standard

Dissolve 0.4389 g. potassium phosphate (Reagent 4) in water, add the required amount of acid and make to 1 liter. Add 1 ml. chloroform as a preservative.

1 ml. = 0.1 mg. P

To prepare a standard curve use aliquots of the standard solution from 0.1 ml. to 0.5 ml., making each aliquot to a total volume of 2 ml. with extracting solution.

References

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PHOSPHORUS

Method No. 2

Principle

Colorimetric method. A phosphomolybdate complex is first formed and then reduced with ferrous sulphate, a much milder reducing agent than that used in Method 1 for phosphorus. The complex molybdenum blue that results is measured colorimetrically.

Reagents

1. Ammonium molybdate-- $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 6.6% solution
2. Sulphuric acid-- H_2SO_4 , 7.5 N (21.3% (v/v))
3. Ferrous sulphate-- $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

Dissolve 5 g. ferrous sulphate in 50 ml. water and add 1 ml. dilute sulphuric acid (Reagent 2). Prepare fresh every two hours.

4. Potassium dihydrogen phosphate-- KH_2PO_4

Procedure

Measure an aliquot of extract into a 50-ml. volumetric flask. Add 5 ml. ammonium molybdate solution (Reagent 1). Add water to bring the volume to approximately 40 ml. Add 5 ml. dilute sulphuric acid (Reagent 2) and mix. Add 4 ml. ferrous sulphate solution (Reagent 3) and mix. Make volume up to 50 ml. and read absorbance immediately in a photoelectric colorimeter using a No. 66 red filter. Set the instrument at zero with a blank determination made with water.

Standard

Dissolve 0.4389 g. potassium phosphate (Reagent 4) in water, add the required amount of acid and make to 1 liter. Add 1 ml. chloroform as a preservative.

1 ml. = 0.1 mg. P

To prepare a standard curve use aliquots of the standard solution from 1 ml. to 5 ml. making each aliquot to a total of 50 ml. with reagents as above.

CALCIUM

Method No. 1

Principle

Turbidimetric method. Calcium is precipitated as a calcium soap which remains in suspension in a finely divided state. It is measured turbidimetrically. See page 4.

Reagents

1. Soap solution

(a) Dissolve 0.6 g. stearic acid and 7.5 ml. oleic acid in 320 ml. of 95% ethyl alcohol, C_2H_5OH . Warm to facilitate solution.

(b) Dissolve 16 g. ammonium carbonate, $(NH_4)_2 CO_3$, in 80 ml. hot water. Mix (a) and (b) and boil 10 minutes. Cool, and add 360 ml. 95% ethyl alcohol, 40 ml. water and 1.6 ml. NH_4OH . Allow to stand 24 hours, filter.

2. Ammoniacal citrate solution

Dissolve 1.5 g. sodium citrate, $Na_3C_6H_5O_7 \cdot 5H_2O$, in water, add 14 ml.

NH_4OH and make to 1 liter. Store in refrigerator and prepare fresh monthly.

3. Precipitated calcium carbonate-- $CaCO_3$

Procedure

Measure 1 ml. of extract into a colorimeter tube. Add 4 ml. ammoniacal citrate (Reagent 2), mix and add 3 ml. soap solution (Reagent 1), allowing solution to flow down the side of the tube to form a layer on top. Mix rapidly and uniformly after the addition of Reagent 1 to all tubes in a given series. Allow to stand 30 minutes. Read the absorbance in a photoelectric colorimeter using a No. 54 green filter. Set the instrument scale at zero with water. The test should not be carried out at temperatures above $28^\circ C$.

Standard

Dissolve 0.4995 g. calcium carbonate (Reagent 3) in acid extracting solution and make to 1 liter with the same solution. Add 1 ml. chloroform as a preservative.

1 ml. = 0.2 mg. Ca

To prepare a standard curve use aliquots of the standard solution from 0.2 ml. to 1.0 ml., making each aliquot to a total volume of 1 ml. with extracting solution.

Reference

Peech, M., and L. English. Rapid microchemical soil tests. Soil Sci. 57: 167-195. 1944.

CALCIUM

Method No. 2

Principle

Volumetric method. Calcium is precipitated as calcium oxalate. The precipitate is dissolved in hot dilute sulphuric acid and titrated with standard potassium permanganate.

Reagents

1. Ammonium oxalate-- $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$, saturated solution.
2. Methyl red indicator
Dissolve 0.5 g. methyl red in 100 ml. 95% alcohol.
3. Acetic acid-- $\text{HC}_2\text{H}_3\text{O}_2$, 20% (v/v)
4. Ammonium hydroxide-- NH_4OH , 20% (v/v)
5. Sulphuric acid-- H_2SO_4 , 20% (v/v)
Add acid to water slowly and with constant stirring. Cool and make up to volume.
6. Sodium oxalate-- $\text{Na}_2\text{C}_2\text{O}_4$, 0.01 N (0.067%)
Before preparing this solution heat the sodium oxalate overnight at 105°C . and cool in a desiccator.
7. Potassium permanganate-- KMnO_4
Stock solution 0.1 N solution (0.316%): Store in a dark bottle.
Standard solution 0.01 N: Dilute 10 ml. stock solution to 100 ml. with water. Prepare fresh before using. Standardize by titrating the standard solution against sodium oxalate solution (Reagent 6) acidified with dilute sulphuric acid. Heat to 80°C . before titrating.

$$\text{Normality of } \text{KMnO}_4 = \frac{\text{g. Na}_2\text{C}_2\text{O}_4}{\text{ml. KMnO}_4 \times 0.067} \quad \text{or}$$

$$\frac{\text{ml. Na}_2\text{C}_2\text{O}_4 \times \text{normality of Na}_2\text{C}_2\text{O}_4}{\text{ml. KMnO}_4}$$

$$1 \text{ ml. } 0.01 \text{ N KMnO}_4 = 0.0002 \text{ g. Ca}$$

Procedure

Measure 10 ml. of extract into a 50-ml. conical centrifuge tube. Add 1 drop methyl red indicator (Reagent 2) and 2 ml. ammonium oxalate (Reagent 1). Then, rotating the tube, add 2 ml. acetic acid (Reagent 3) and mix. Make solution slightly alkaline by the addition of NH_4OH (Reagent 4) and then slightly acid with a few drops of acetic acid (Reagent 3). Allow to stand 4 hours. Centrifuge, discard the supernatant, drain off the last few drops and wipe the lip of the tube. Add 4 ml. dilute NH_4OH (Reagent 4), mix, centrifuge and discard the supernatant, draining as before.

Add 10 ml. hot dilute H_2SO_4 (Reagent 5) taking care to drop the acid directly on the precipitate. Place tube in water bath at 80°C . and titrate with standard potassium permanganate (Reagent 7) using a microburette.

References

McCance, R.A., and H.L. Shipp. The chemistry of flesh foods and their losses on cooking. Med. Res. Council Spec. Series No. 187. 1933.

MAGNESIUM

Method No. 1

Principle

Colorimetric method. This method makes use of the adsorption of the dye Thiazole yellow on colloidal magnesium hydroxide. The addition of a stabilizing colloid, in this case polyvinyl alcohol, prevents coagulation of the particles. See page 4.

Reagents

1. Hydroxylamine hydrochloride-- $\text{NH}_2\text{OH} \cdot \text{HCl}$, 1 % solution

Prepare fresh weekly.

2. Compensating solution

Calcium chloride -- CaCl_2	1.40 g.
Aluminum sulphate $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$	0.37 g.
Manganous sulphate $\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.16 g.
Sodium phosphate -- $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$	0.70 g.
tribasic	
Copper sulphate -- $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.059 g.
Hydrochloric acid -- HCl	5.00 ml.

Weigh the salts into a dry container. Add a few ml. water, then add the 5 ml. HCl . Add more water to dissolve the salts and make the solution to a volume of 1 liter. Do not apply heat to aid in solution.

3. Polyvinyl alcohol, Du Pont, Elvanol, Grade 71-24, 2 % solution

Mix 20 g. with about 400 ml. water, heat to about 90°C . on water bath and stir until dissolved. Cool, dilute to 1 liter and filter if not clear. Keep in refrigerator. Prepare fresh each month.

4. Mixed reagent

Mix equal parts of 1, 2, and 3 just before use.

5. Thiazole yellow or Titan yellow

Stock solution: Dissolve 0.5 g. thiazole yellow in 100 ml. 50 % ethyl alcohol. Store in a dark bottle. Solution keeps indefinitely.

Reagent: 0.02 % solution, dilute 2 ml. stock solution to 50 ml. with 50 % (v/v) ethyl alcohol and store in dark bottle. Prepare fresh weekly from the stock solution.

6. Sodium hydroxide- NaOH , 10 N solution (40 %)

7. Magnesium sulphate-- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

Procedure

Measure 5 ml. of extract into a colorimeter tube. Add 3 ml. mixed reagent (Reagent 4) and mix. Add 1 ml. thiazole yellow (Reagent 5) and mix. Add 2 ml. sodium hydroxide (Reagent 6) and mix. Allow to stand 10 minutes in a con-

stant-temperature water bath at 28°C. Read absorbance in a photoelectric colorimeter using a No. 54 green filter. Set the instrument scale at zero with water.

Standard

Dissolve 2.559 g. magnesium sulphate (Reagent 7) in water and make to 250 ml. Dilute 5 ml. of this solution and the required amount of acid to 1 liter. 1 ml. = 5 micrograms Mg.

To prepare a standard curve use aliquots of the standard solution from 1 ml. to 5 ml. making each aliquot to a total volume of 5 ml. with extracting solution.

References

- Peech, M., and L. English. Rapid microchemical soil tests. Soil Sci. 57: 167-195. 1944.
- Young, H. Y., and R. F. Gill. Determination of magnesium in plant tissue with thiazole yellow. Anal. Chem. 23: 751-754. 1951.

MAGNESIUM

Method No. 2

Principle

Colorimetric method. In an alkaline solution from which calcium and iron have been removed magnesium is precipitated as magnesium ammonium phosphate. The precipitate is dissolved in acid and the amount of phosphorus is determined colorimetrically by one of the standard procedures. Magnesium is then determined by calculation.

Reagents

1. Ammonium oxalate-- $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$, saturated solution

2. Methyl red indicator

Dissolve 0.5 g. methyl red in 100 ml. 95% ethyl alcohol.

3. Ammonium phosphate-- $(\text{NH}_4)_2\text{HPO}_4$, 2% solution

4. Ammonium hydroxide-- NH_4OH , 10% (v/v) solution

5. Hydrochloric acid-- HCl , 0.1 N. (0.89% v/v)

6. Molybdic acid solution

Dissolve 25 g. ammonium molybdate in 300 ml. water without heating. Dilute 75 ml. H_2SO_4 to 200 ml. with water and add to the ammonium molybdate solution. Store in brown bottle.

7. Hydroquinone-- 2% solution

Add 1 drop H_2SO_4 for each 100 ml. Discard when solution starts to become brown.

8. Sodium sulphite-- Na_2SO_3 , 20% solution

Prepare fresh weekly.

Note: Test Reagents 6, 7, 8 by mixing. No blue or green color should appear.

9. Potassium dihydrogen phosphate-- KH_2PO_4

Procedure

Measure 10 ml. of extract into a 15-ml. graduated centrifuge tube. Add 1 drop methyl red indicator (Reagent 2) Neutralize solution with NH_4OH . Add 1 ml. ammonium oxalate (Reagent 1) and make solution to a volume of 15 ml. with water. Mix and allow to stand overnight. Centrifuge for 10 minutes and discard the precipitate.

Measure 1 ml. of the supernatant liquid from above into a 15-ml. centrifuge tube. Add 3 ml. water, 1 ml. ammonium phosphate (Reagent 3) and 2 ml. NH_4OH . Mix and allow to stand overnight. Centrifuge for 7 minutes, discard the supernatant liquid, add 5 ml. dilute NH_4OH (Reagent 4), mix, centrifuge for 7 minutes, discard the supernatant liquid. Dry the precipitate by placing the tube in a container of hot water.

Add 1 ml. dilute HCl (Reagent 5) and 5 ml. water to dissolve the precipitate. Add 1 ml. molybdic acid solution (Reagent 6), 0.5 ml. hydroquinone

(Reagent 7), and 0.5 ml. sodium sulphite (Reagent 8). Mix, allow to stand 30 minutes. Transfer solution to a colorimeter tube and read absorbance in a photoelectric colorimeter using a No. 66 red filter. Set the instrument scale at zero with water.

Standard

Dissolve 0.4389 g. potassium phosphate (Reagent 9) in water and make to a volume of 1 liter.

1 ml. = 0.1 mg. P = 0.0784 mg. Mg

To prepare a standard curve use aliquots of the standard solution from 0.1 to 0.5 ml. treating each aliquot as directed above beginning "add 1 ml. dilute HCl...".

References

- Holzappel, C.R. Mineral metabolism. 27. Modifications of the methods used at Onderstepoort for the determination of (a) magnesium and calcium, (b) potassium in grass extracts. Onderstepoort J. Veterinary Sci. and An. Ind. 2: 115-122. 1934.
- McCance, R.A., and H.L. Shipp. The chemistry of flesh foods and their losses on cooking. Med Res. Council. Spec. Rept. Series No. 187. 1933.

CALCIUM AND MAGNESIUM

Principle

Volumetric method. In alkaline solution in the absence of heavy metal ions, calcium and magnesium will form complexes with versene (ethylenediamine tetra-acetic acid). When both are present and the solution is titrated with versene, all the calcium will be complexed first and the end point of this reaction can be determined with the indicator Cal-Red. The end point of the reaction when both calcium and magnesium have been complexed with versene is determined with the indicator Eriochrome black T. Magnesium concentration is calculated from the difference between the two titrations.

Reagents

1. Eriochrome black T indicator

Triturate 0.2 g. Eriochrome black T and 50 g. potassium chloride, KCl, with a mortar and pestle until all the dye is evenly distributed on the surface of the KCl crystals.

2. Cal-Red indicator

Triturate 0.5 g. Cal-Red (Scientific Service Laboratories Inc. P.O. Box No. 175, Dallas 21, Texas, U.S.A.) and 50 g. sodium sulphate, Na_2SO_4 , with a mortar and pestle until the dye is evenly distributed.

3. Standard calcium solution

Dry a quantity of pure calcium carbonate, CaCO_3 , at a temperature of 250°C . Dissolve 1.000 g. of the dried salt in 250 ml. 0.1 N HCl. Add 40 ml. 0.1 N NaOH. Dilute solution to 1 liter. Solution is 0.02 N.

4. Standard magnesium solution

Dissolve 2.03 g. magnesium chloride, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, in water and dilute to 1 liter. Solution is 0.02 N.

5. Versene solution

Dissolve 3.0 g. di-sodium versenate (di-sodium ethylenediamine tetra-acetic acid $2\text{H}_2\text{O}$) in water and dilute to 1 liter. Store in polyethylene bottle if possible. Standardize against standard calcium solution (Reagent 3) using Eriochrome black T indicator (Reagent 1).

1 ml. 0.02 N EDTA = 0.4008 mg. Ca

1 ml. 0.02 N EDTA = 0.2432 mg. Mg

6. Buffer solution

Add 55 ml. HCl to 400 ml. water and mix. Add 310 ml. monoethanolamine (Eastman, white label) slowly with stirring. Cool. Titrate 50 ml. standard magnesium solution (Reagent 4) with versene solution (Reagent 5) using 1 ml. monoethanolamine-HCl solution as buffer. Use procedure described below for calcium plus magnesium. Add 50 ml. standard magnesium solution (Reagent 4) to the exact amount of versene solution (Reagent 5) required to complex all the magnesium as calculated from the above titration. Pour this mixture into the monoethanolamine-HCl solution prepared above. Dilute to 1 liter.

7. Potassium cyanide-- KCN. Caution: Poison!
8. Hydroxylamine hydrochloride-- $\text{NH}_2\text{OH} \cdot \text{HCl}$
9. Composite solution
Reagents 7 and 8 may be combined in solution form by dissolving 2 g. of each in water and diluting the mixture to 100 ml. This solution must be prepared fresh daily.
10. Potassium hydroxide solution-- KOH, 8 N (44.98%)

Procedure

A. Calcium plus Magnesium

Measure an aliquot of slightly acid plant extract containing from 0.002 g. - 0.005 g. Ca into a titration container. Dilute to about 50 ml. with water. Add 5 ml. buffer solution (Reagent 6). Add about 100 mg. each of potassium cyanide (Reagent 7) and hydroxylamine hydrochloride (Reagent 8) or add 5 ml. of composite solution (Reagent 9). Mix. Add 0.1 g. Eriochrome black T indicator (Reagent 1). Use a calibrated measuring spoon for convenience. Titrate to the pure blue end point with versene solution (Reagent 5) and with a light under the container. Make a blank titration using water and apply the appropriate correction.

B. Calcium

Measure duplicate aliquots of extract into titration containers. Dilute each to about 50 ml. with water. To the first container add 4 ml. KOH solution (Reagent 10). Mix. If a precipitate forms allow to stand 3-5 minutes and shake occasionally. Add about 100 mg. each of potassium cyanide, (Reagent 7) and hydroxylamine hydrochloride (Reagent 8), or add 5 ml. of composite solution (Reagent 9). Mix. Add 0.1 g. or 1 measuring spoonful of Cal-Red indicator (Reagent 2) and titrate solution to the pure blue end point with versene solution (Reagent 5) and with a light under the container. Now to the second aliquot of extract add the amount of versene solution (Reagent 5) used in the first titration less 1 ml. Add 4 ml. KOH solution (Reagent 10). Mix. Complete the titration procedure as with the first aliquot, but use the second titration as the correct value. Make a blank titration using water and apply the appropriate correction.

C. Magnesium

The volume of versene equivalent to the magnesium in the extract is found by subtracting the titration value found in B (calcium) from the titration value found in A (calcium plus magnesium) for equal aliquots.

D. Alternative Procedure

As an alternative to the three steps outlined above, the following procedure may be used.

- (1) Complete the titration for calcium plus magnesium as described in A.
- (2) Measure an equal aliquot into a 50-ml. centrifuge tube and precipitate the calcium as calcium oxalate according to the procedure described for

Calcium, Method 2, page 24 . The combined supernatant and washings are then titrated with versene solution (Reagent 5) according to the procedure described in A above for calcium and magnesium. This titration will give the volume of standard versene equivalent to the magnesium in the aliquot. (3) The volume of versene equivalent to the calcium in the extract is found by subtracting the titration value found in (2) (magnesium) from the titration value found in (1) (calcium plus magnesium) for equal aliquots.

References

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- Maier, R. H. A new sensitive indicator for semi-micro chelometric titrations. Nature 183: 461-2. 1959.

CHLORINE

Principle

Volumetric method. Chlorine in acid solution is precipitated as silver chloride with a measured amount of silver nitrate. The silver nitrate remaining after precipitation is titrated with ammonium thiocyanate using as an indicator ferric alum which gives a pink end point.

Reagents

1. Sodium carbonate-- Na_2CO_3 , 5% solution
2. Nitric acid-- HNO_3 , 20% (v/v).
3. Nitric acid-- HNO_3 , concentrated, colorless
4. Silver nitrate-- AgNO_3 , 0.01 N solution (1.699%)
Store in dark bottle.

5. Nitrobenzene
6. Ferric alum indicator-- $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$

Prepare a saturated solution of ferric ammonium sulphate without heating. Filter and bleach the reddish-brown color to a straw yellow by addition of colorless HNO_3 .

7. Ammonium thiocyanate-- NH_4SCN , 0.01 N solution.

Since ammonium thiocyanate is deliquescent and it is difficult to prepare an exactly 0.01 N solution directly, the following procedure is used. Dissolve 9 g. in water and dilute to 1 liter. To 10 ml. 0.1 N AgNO_3 , add 5 drops HNO_3 and 1 ml. ferric alum indicator. Titrate to a pink end point with the ammonium thiocyanate. A 0.01 N solution may be obtained by diluting to 1 liter ten times the volume of thiocyanate solution used in the titration.

Preparation of Extract for Total Chlorine

Weigh 5.000 g. of ground dried tissue into a silica dish. Add 20 ml. sodium carbonate solution (Reagent 1) from a pipette, making sure that all the tissue has been moistened. Heat on a water bath until the tissue is dry, then ignite in a muffle furnace at 500°C . for 1 hour. Cool and add about 25 ml. boiling water. Break up the carbonized sample with a stirring rod and filter into a 200-ml. volumetric flask. Wash well with hot water. Place filter paper with residue in original silica dish and return to the muffle furnace. Ignite at 500°C . until a grayish-white ash is obtained. Cool, moisten with water, cautiously add dilute HNO_3 (Reagent 2) until effervescence ceases. Filter into the original 200-ml. volumetric flask. If a precipitate forms in the flask that will not redissolve upon shaking, add more dilute HNO_3 until a clear solution is obtained. Make the solution up to volume with water.

Procedure

Measure 2 ml. of extract into a 50-ml. Erlenmeyer flask. Add 3 drops HNO_3 (Reagent 3) and mix. Add 5 ml. 0.01 N silver nitrate (Reagent 4) and shake to coagulate the precipitate. Add 4 drops nitrobenzene and shake again. Add 1 ml. ferric alum indicator (Reagent 6) and titrate with 0.01 N ammonium thiocyanate to the first pink color. If the pink color is obtained on addition of the first drop, a smaller aliquot of extract must be used. If the thiocyanate titration is greater than 4 ml., a larger aliquot of extract must be used.

Calculation

$$\frac{355 \times (5.00 - \text{ml. thiocyanate})}{\text{ml. aliquot of extract}} = \text{p. p. m. Cl in extract}$$

(1 ml. of 0.1 N AgNO_3 = 0.00355 g. Cl)

References

Caldwell, John R., and Harvey V. Moyer. Determination of chloride. A modification of the Volhard method. Ind. Eng. Chem. Anal., Ed. 7: 38-39. 1955.

SULPHUR

Principle

Gravimetric method. Sulphur is precipitated from an acid solution of the ash as the barium salt which is ignited and measured gravimetrically.

Reagents

1. Magnesium nitrate-- $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 96.2 % solution
2. Barium chloride-- BaCl_2 , 10 % solution

Procedure

Weigh 1.000 g. of dried ground sample into a 400-ml. pyrex beaker. Add 7.5 ml. magnesium nitrate solution (Reagent 1) and rotate beaker to ensure that all material is brought into contact with the solution. Heat carefully on a moderate hot plate until no further liquid remains and the evolution of brown fumes ceases. Transfer immediately to a muffle furnace at about 350°C . Heat for 30 minutes or until a completely white residue remains. Remove beaker and cool. Add water and HCl in excess. Boil solution, cool, filter into a 200-ml. volumetric flask, washing the filter paper thoroughly with water. Make solution to volume with water.

Measure a 50-ml. aliquot of the prepared solution into a 250-ml. beaker. Heat to boiling and add by drops from a pipette 10 ml. of barium chloride solution (Reagent 2). Stir constantly. Cover beaker with a watch glass and continue boiling for a few minutes. Cool and allow to stand several hours. Filter solution through a high-grade filter paper (No. 589 (blue) S. and S. or No. 42 Whatman). Transfer precipitate quantitatively to the filter using a rubber policeman if necessary and wash six times with boiling water. Transfer filter paper with precipitate to an ignited and weighed porcelain crucible. Dry and ignite in muffle furnace at 500°C . for 1 hour. Cool in desiccator and weigh.

Wt. of precipitate $\times 0.1374$ = g. S. in aliquot.

IRON

Principle

Colorimetric method. Ferric iron in acid solution reacts with potassium thiocyanate to form the red compound, ferric thiocyanate, which is extracted with an organic solvent and measured colorimetrically.

Reagents

1. Isoamyl alcohol-- $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{OH}$
2. Potassium thiocyanate--KSCN, 20 % solution
3. Ferrous ammonium sulphate-- $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$
4. Sulphuric acid-- H_2SO_4 , 5% (v/v).

Add 5 ml. H_2SO_4 with stirring to 95 ml. water.

Procedure

Measure an aliquot of extract into a 50-ml. boiling tube. Make volume up to 20 ml. with water. Add 4 drops HNO_3 and heat tube in a boiling water bath for 40 minutes. Cool and transfer solution to a 125-ml. separatory funnel. Add 10 ml. amylalcohol (Reagent 1) and 5 ml. potassium thiocyanate (Reagent 2.). Shake vigorously 60 times. Allow to settle about 5 minutes. Discard the now colorless bottom aqueous layer. Transfer the colored alcohol layer to a 15-ml. centrifuge tube. Centrifuge for 3 minutes at 1000 g. Transfer alcohol layer to a colorimeter tube and read absorbance in a photoelectric colorimeter using a No. 54, green filter. Set the instrument scale at zero with amyl alcohol. (Reagent 1)

As an alternative to the above procedure, the following may be used for very small samples or for extracts containing small amounts of iron. Measure an aliquot of extract containing less than 15 micrograms of iron into a boiling tube. Make volume up to 5 ml. with water. Add 1 drop HNO_3 and heat in boiling water bath. Cool and transfer solution to 30-ml. separatory funnel. Add 3 ml. amyl alcohol (Reagent 1) and 2 ml. potassium thiocyanate (Reagent 2); shake vigorously 60 times. Allow to settle 5 minutes or longer. Transfer with a pipette a portion of the colored alcohol layer to a special flat-bottomed colorimeter tube and read absorbance as directed above.

Standard

Dissolve 3.514 g. ferrous ammonium sulphate (Reagent 3) in a minimum quantity of water. Add 100 ml. dilute sulphuric acid (Reagent 4) and mix. Add 17 ml. HNO_3 . Allow to stand overnight, then make volume to 1 liter. Dilute 10 ml. of this solution to 1 liter with water.

1 ml. = 0.005 mg. Fe

To prepare a standard curve use aliquots of the standard solution from 2 ml. to 10 ml. making each aliquot to 20 ml. in a separatory funnel and omitting

oxidation by boiling as outlined in the above procedure.

To prepare a standard curve for the alternative procedure use aliquots of the standard solution from 0.5 ml. to 3 ml. making each aliquot to 5 ml. in a 30-ml. separatory funnel and omitting oxidation by boiling.

References

McCance, R.A., and H.L. Shipp. The chemistry of flesh foods and their losses on cooking. Med. Res. Council Spec. Rept. Series No. 187. 1953.

MANGANESE

Principle

Colorimetric method. Manganese in an acid solution from which chloride has been eliminated is oxidized to permanganate with potassium periodate. The purple permanganate is measured colorimetrically.

Reagents

1. Phosphoric acid-- H_3PO_4 . 9% (v/v)
2. Potassium periodate-- KIO_4
3. Potassium permanganate-- KMnO_4
4. Sodium bisulphite-- NaHSO_3

Procedure

Measure an aliquot of extract into a 250-ml. beaker. Evaporate to dryness cautiously. Add 2 ml. HNO_3 and again evaporate to dryness. Repeat this treatment twice. Add 20 ml. dilute phosphoric acid (Reagent 1). Cover the beaker with a watch glass and heat solution to boiling. Add approximately 0.3 g. of potassium periodate (Reagent 2). Keep solution near boiling for about 3 minutes to allow for full color development. Cool, transfer to a 25-ml. volumetric flask and make to volume with water. Centrifuge a portion of this solution if necessary and transfer to a colorimeter tube. Read absorbance in a photoelectric colorimeter using a No. 54 green filter. Set the instrument scale at zero with 20 ml. dilute phosphoric acid (Reagent 1) diluted to 25 ml. with water.

Standard

Stock solution: Dissolve 0.144 g. potassium permanganate (Reagent 3) in 100 ml. water. Add 5 ml. dilute phosphoric acid (Reagent 1), a small amount of sodium bisulphite (Reagent 4) and stir until the purple color disappears. Boil solution to expel the excess SO_2 . Cool, add 90 ml. concentrated phosphoric acid and dilute to 1 liter with water.

1 ml. = 0.05 mg. Mn

Standard solution: Measure 20 ml. of stock solution into a 250-ml. beaker. Heat solution to boiling. Add approximately 0.3 g. of potassium periodate (Reagent 3). Keep solution near boiling for about 3 minutes to allow for full color development. Cool and dilute to 100 ml. with dilute phosphoric acid (Reagent 1).

1 ml. = 0.01 mg. Mn

To prepare a standard curve use aliquots of 4 ml. to 20 ml. of standard solution, making each aliquot to a volume of 20 ml. with dilute phosphoric acid (Reagent 1) and to a final volume of 25 ml. with water.

References

- Willard, H. H. , and L. H. Greathouse. The determination of manganese by oxidation with periodate. J. Am. Chem. Soc. 39: 2366-2369. 1917.
- Peech, M. Determination of exchangeable bases in soil. Ind. and Eng. Chem. Anal. Ed. 13: 436-441. 1941.

COPPER

Principle

Colorimetric method. Copper in an ammoniacal solution reacts quantitatively with sodium diethyl-dithiocarbamate to form a colored complex. The colored substance is extracted with an organic solvent and measured colorimetrically.

Reagents

1. Ammonium hydroxide-- NH_4OH , 50 % (v/v) solution
2. Citric acid-- $\text{C}_6\text{H}_8\text{O}_7$, 15 % solution
3. Phenolphthalein indicator

Dissolve 1 g. in 100 ml. 95% ethyl alcohol.

4. Sodium diethyl- dithiocarbamate-- $(\text{C}_2\text{H}_5)_2\text{NCS}_2\text{Na}$

Dissolve 0.5 g. in water. Dilute to 500 ml. and filter. Transfer to a separatory funnel, add about 10 ml. carbon tetrachloride, CCl_4 , and shake well. Allow to settle and discard the carbon tetrachloride layer. Filter the water layer and store for use. Prepare fresh every two weeks.

5. Isoamyl alcohol-- $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{OH}$
6. Copper sulphate-- $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

Procedure

Measure 5 ml. of extract into a small separatory funnel. Add 5 ml. citric acid (Reagent 2). Add two drops of phenolphthalein indicator (Reagent 3). Titrate to a definite pink color with dilute ammonium hydroxide (Reagent 1). Add 10 ml. sodium diethyl-dithiocarbamate solution (Reagent 4) and 10 ml. isoamyl alcohol (Reagent 5). Shake vigorously 60 times. Allow to settle about 5 minutes. Discard the now colorless bottom aqueous layer. Transfer the colored alcohol layer to a 15-ml. centrifuge tube. Centrifuge for 3 minutes at 1000 g. Transfer the alcohol layer to a colorimeter tube and read absorbance in a photoelectric colorimeter using a No. 42 blue filter. Set the instrument scale at zero with amyl alcohol. (Reagent 5)

Standard

Dissolve 1.186 g. copper sulphate (Reagent 6) in water and make solution to a volume of 1 liter. Dilute 10 ml. of this solution to 100 ml. with water.
1 ml. = 0.03 mg. Cu

To prepare a standard curve use aliquots of this solution from 0.4 ml. to 2.0 ml. making each aliquot to a volume of 5 ml. with water.

References

Callan, T., and J.A.R. Henderson. A new reagent for the colorimetric determination of minute amounts of copper. Analyst 54: 650-653. 1929.

ZINC

Principle

Polarographic method. A purified solution of zinc is electrolyzed at a controlled pH in a cell equipped with a dropping-mercury electrode. A unidirectional polarizing potential is applied which is increased at a uniform rate. Ions of zinc are discharged at the polarized electrode, and the magnitude of the current flowing is directly proportional to the concentration of discharging ions thus giving a measure of zinc in solution.

Reagents

1. Hydrochloric acid-- HCl , 54 % (v/v)
2. Sodium acetate-- $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, 20 % solution
3. Gelatin, 0.1 % solution
4. Mercury

Purify by washing with dilute HNO_3 (1 + 3) and water.

5. Zinc acetate standard solution

Dissolve 0.8392 g. $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ in water and make to a volume of 1 liter. Dilute 10 ml. of this solution to 500 ml.

1 ml. = 5 micrograms Zn

6. Nitrogen gas-- N_2

Purify commercial nitrogen by passing it through two gas-washing towers. In the first tower place a solution of NH_4OH , 50 % (v/v) saturated with ammonium chloride, NH_4Cl , and containing copper turnings. In the second tower place dilute H_2SO_4 , 50 % (v/v).

7. Magnesium acetate solution

Dissolve 4.054 g. $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$ in 50 ml. water and make to a volume of 1 liter with ethyl alcohol, $\text{C}_2\text{H}_5\text{OH}$.

Procedure

Weigh 1.000 g. of dried, ground sample into a small silica dish. Transfer to a cold muffle furnace and raise the temperature gradually to 500°C . over a period of 30 minutes. Continue ignition at this temperature for 30 minutes. For certain materials such as grain full recovery of zinc is not obtained from ash prepared in this manner. For the analysis of such samples add 5 ml. Reagent 7 to the tissue in the silica dish before ignition. Moisten the ash with water, add 1.5 ml. dilute HCl (Reagent 1) and evaporate to dryness on a boiling water bath. Cool, add 5 ml. water and 0.1 ml. dilute HCl (Reagent 1). Transfer solution with washing to a 15-ml. graduated centrifuge tube and add 0.6 ml. sodium acetate solution (Reagent 2) and 0.25 ml. of gelatin solution (Reagent 3). Make solution to a volume of 10 ml. with water. Allow tube to stand for a few minutes until precipitate begins to flocculate, then centrifuge for 5 minutes at 1000 g.

Measure 4 ml. of extract into the electrolysis cell of the polarograph.

Add sufficient mercury to cover the bottom of the cell. Remove oxygen from the solution by bubbling with purified nitrogen for 10 minutes. Adjust the polarograph so that all circuits are properly balanced. Adjust temperature of the test solution to approximately 25°C. Connect the circuit through the electrolysis cell and allow the current to flow over a voltage range of -0.6 to -1.4 volts. Disconnect circuit through electrolysis cell. Add 2 ml. zinc standard solution (Reagent 5). Bubble nitrogen through the solution for 5 minutes. Connect the circuit again through the cell and allow the current to flow over the same voltage range as above. The amplitude of the wave traced on the automatic recording chart is proportional to the concentration of zinc in the test solution in the electrolysis. A blank determination should be made on the reagents using the same procedure, and appropriate correction applied.

Calculation formula

$$\left(\frac{10A}{\frac{3}{2}B - A} - \text{blank} \right) \times \frac{10}{4} = \text{p. p. m. Zn in sample}$$

where A = amplitude of curve from solution of sample

B = amplitude of curve from solution of sample + standard

References

Bibliography of the polarized dropping - mercury electrode. Bibliography E-94(1), 1940. Leeds and Northrup Co. 4907 Stenton Ave., Philadelphia, Pa., U.S.A.
The Electrochemograph Bulletin E-94(1), 1942. Leeds and Northrup Co., 4907 Stenton Ave., Philadelphia, Pa., U.S.A.
Kolthoff, I. M., and J. J. Lingane. Polarography, Volumes 1 and 2. 1952. Interscience Publishers, New York.

BORON

Principle

Colorimetric method. In acid solution boron reacts with turmeric to form an orange-colored complex which is measured colorimetrically.

Reagents

1. Calcium hydroxide-- Ca(OH)_2
2. Hydrochloric acid - oxalic acid solution
Dissolve 4 g. oxalic acid $\text{H}_2\text{C}_2\text{O}_4$, in 20 ml. water and add 5 ml. HCl . Warm before using in order to keep oxalic acid in solution.
3. Turmeric solution
Dissolve 0.4 g. turmeric powder in 40 ml. of 95% ethyl alcohol. Prepare fresh daily and allow to stand 18 hours before use.
4. Ethyl alcohol, 95%
5. Boric acid-- H_3BO_3

Procedure

Weigh 25 mg. of dried ground tissue into a small silica dish. Add 20 mg. of calcium hydroxide (Reagent 1) and mix well with sample, using a small metal spatula. Place silica dish in a muffle furnace at a temperature of 250-300°C. Raise the temperature to 650°C. and maintain this temperature for 15 minutes. Remove the dish, cool and add 1 ml. of hydrochloric acid--oxalic acid solution (Reagent 2). Ensure that all the residue is wet with this reagent. Add 2 ml. of turmeric solution (Reagent 3). Mix by rotating the dish. Evaporate to dryness and bake for two hours on a water bath kept at a constant vapor temperature of 53°C - 55°C. Cool, and add about 20 ml. alcohol (Reagent 4) and filter into a 50-ml. volumetric flask. Wash all the color from the dish and filter paper with alcohol. Make up to volume, transfer a portion to a colorimeter tube and read the absorbance on a photoelectric colorimeter using a No. 54 green filter. Set the instrument scale at zero with water.

Note: Pyrex glass must be avoided throughout this determination, and Reagents 2 and 3 and all standard solutions must be stored in boron-free glass or polyethylene containers.

Filter papers must be well washed with hot HCl (1 + 1), hot water and alcohol. All dishes, flasks, and funnels must be washed with hot water immediately before using.

Standard

Dissolve 0.2858 g. boric acid in water and make to a volume of 1 liter. Dilute 10 ml. of this solution to 1 liter with water.

1 ml. = 0.0005 mg. B

To prepare a standard curve use aliquots of this solution from 1 ml. to 8 ml. Add 20 mg. calcium hydroxide (Reagent 1) to each aliquot, evaporate to dry-

ness on a steam bath and follow the procedure outlined above beginning at "add 1 ml. of Reagent 2". Blanks of calcium hydroxide alone must also be analyzed.

References

- Yoe, J.H. Determination of boric acid by turmeric paper. Photometric chemical analysis, Vol. 1 - Colorimetry. 1928. John Wiley and Sons, New York.
- Naftel, J.A. Colorimetric microdetermination of boron. Ind. & Eng. Chem. Anal. Ed. 11: 407-409. 1939.

MOLYBDENUM

Principle

Colorimetric method. Molybdenum in acid solution reacts with ammonium thiocyanate to form a colored compound which is measured colorimetrically.

Reagents

1. Ammonium thiocyanate-- NH_4SCN , 20 % solution
2. Stannous chloride-- SnCl_2 , 10 % solution
3. Amyl alcohol - amyl acetate mixture, 1 + 1 mixture
4. Ammonium molybdate -- $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$
5. Perchloric acid-- HClO_4 , 72 %

Note: Special precautions must be observed throughout this procedure to avoid contamination. Contact of the plant material with metal must be avoided if possible during preparation of the sample. The dried sample must be ground with an agate mortar and pestle. Distilled water redistilled from pyrex glass must be used throughout. All glassware must be washed with HCl , then rinsed first with distilled water and finally with redistilled water. Reagents amyl alcohol, hydrochloric acid and nitric acid must be redistilled before use. All reagents should be tested by running a blank determination with reagents only.

Procedure

Weigh 5.000 g. of dried ground sample into a 300-ml. Kjeldahl flask. Add 50 ml. redistilled HNO_3 . Allow to stand overnight. Heat slowly until the first fumes have been driven off, then more vigorously for about 30 minutes until the volume of solution is reduced to about 20 ml. Cool, add 5 ml. perchloric acid (Reagent 5) and 2 ml. H_2SO_4 .

Heat gently until frothing ceases and then more vigorously after the organic matter has been oxidized, to remove perchloric acid completely. Sulphuric acid which remains should condense on the bulb of the flask. Add a little hot water and maintain at a temperature of about 60°C . for 15 minutes. Cool and filter into a 50-ml. volumetric flask. Add 3 ml. HCl and make up to a volume of 50 ml. with water. Measure a 10-ml. aliquot into a 125-ml. separatory funnel. Add 5 ml. HCl and 3 ml. of ammonium thiocyanate (Reagent 1) and mix. Then add 3 ml. of stannous chloride (Reagent 2) to discharge the red ferric thiocyanate color. More stannous chloride must be added if the red color persists. Add 6 ml. of Reagent 3 and shake vigorously then allow to settle for 5 minutes. Pipette the colored amyl alcohol-amyl acetate layer into a 15-ml. centrifuge tube. Centrifuge for 3 minutes. Transfer the amyl alcohol-- amyl acetate to a colorimeter tube and read the absorbance in a photoelectric colorimeter using a No. 42 blue filter. Set the instrument scale at zero with amyl alcohol--amyl acetate (Reagent 3).

Standard

Dissolve 0.184 g. ammonium molybdate (Reagent 4) in water and make solution to a volume of 100 ml. with water. Dilute 5 ml. of this solution to 500 ml. with water.

1 ml. = 0.01 mg. Mo

To prepare a standard curve use aliquots of this solution from 0.5 ml. to 2.0 ml. and make each aliquot to a volume of 50 ml. with HCl and water as directed above.

References

- Barshad, I. Molybdenum determination in plant material. Modification of thiocyanate stannous chloride method. Anal. Chem. 21: 1148-1150. 1949.
- Ward, F. N. Determination of molybdenum in soils and rocks. Anal. Chem. 23: 788-791. 1951.

COBALT

Principle

Colorimetric method. In acid solution cobalt forms a colored complex with 1-nitroso-2-naphthol which is extracted with carbon tetrachloride and measured colorimetrically.

Reagents

1. Sodium hydroxide-- NaOH, 0.1 N, (0.4%)
2. 1-nitroso-2-naphthol-- 0.05% solution in 0.1 N NaOH.
Recrystallize the salt from water before use.
3. Hydrogen peroxide-- H_2O_2 , 3% solution
4. Urea-- NH_2CONH_2 , 30% solution.
Prepare fresh before use.
5. Carbon tetrachloride-- CCl_4
6. Sodium acetate-- $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, 50% solution
7. Ethyl alcohol-- $\text{C}_2\text{H}_5\text{OH}$, 95%
8. Wash solution-- 1 volume 95% ethyl alcohol + 1 volume 0.1 N NaOH (Reagent 1)
9. Cobalt sulphate-- $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$
10. Hydrofluoric acid-- HF

Procedure

Weigh 5.000 g. of dried ground sample into a platinum dish. Place in a muffle furnace and raise the temperature gradually to 500°C . Maintain at this temperature for 15 hours. Cool, moisten the ash with water, add 2-4 ml. perchloric acid and 5 ml. hydrofluoric acid (Reagent 10) and heat on a steam bath for 1 hour. Transfer to a hot plate and heat until all perchloric acid fumes are eliminated. Place in a muffle furnace not above 200°C . and raise the temperature to 600°C . Maintain at this temperature for 1 hour.

Cool, add 10 ml. water and 2.5 ml. HCl, cover with a watch glass and heat gently. Transfer contents to a 250-ml. beaker. Rinse platinum dish with water to make a total volume of 100 ml. Add 5 ml. sodium acetate solution (Reagent 6) and enough HCl to adjust the pH to approximately 1.5. Add a few drops of hydrogen peroxide (Reagent 3) and 10 ml. urea solution (Reagent 4). Boil until pH has risen to above 3.5 but not above 4.5.

Cool, adjust pH to 4.0 with sodium acetate (Reagent 6) or HCl and filter into a small separatory funnel. Refilter if the filtrate is not clear. Wash filter paper with hot water. To the filtrate in the separatory funnel add 5 ml. of 1-nitroso-2-naphthol solution (Reagent 2), mix, and allow to stand for 1 hour. Extract five times with carbon tetrachloride using 10 ml. for the first extraction and 5 ml. for each of the four following extractions. Draw off the carbon tetrachloride layer each time into a separatory funnel containing 10 ml. HCl. Also drop any film at the interface into the acid, wash each extract successively in each of six separatory funnels containing the following:

1. 10 ml. HCl
2. 20 ml. water
3. 10 ml. wash solution (Reagent 8)
4. 10 ml. wash solution (Reagent 8)
5. 20 ml. water
6. 20 ml. water

Shaking should be vigorous but need not exceed one half minute by which time the carbon tetrachloride layer clears. After the first two extracts have passed through funnel No. 4, add the wash from this funnel to that in funnel No. 3 and replace it with fresh alcoholic sodium hydroxide (Reagent 8).

Evaporate the combined carbon tetrachloride extracts in a beaker on a water bath to about 2 ml., transfer to a 5-ml. volumetric flask and make up to volume with carbon tetrachloride. Measure the absorbance on a Beckman spectrophotometer at a wave length of 400 millimicrons.

If a photoelectric colorimeter is to be used for determining absorbance, a sample of 20 g. must be ashed to give sufficient cobalt to produce a measurable amount of color, and the final volume of carbon tetrachloride is made up to 10 ml. A No. 42 blue filter is used in measuring the absorbance and the instrument scale is set at zero with carbon tetrachloride.

Standard

Heat $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ (Reagent 9) in an oven at 250°C . for 8 hours to constant weight. Dissolve 0.263 g. of the resultant CoSO_4 in 50 ml. redistilled water containing 1 ml. H_2SO_4 . Make to a volume of 1 liter with redistilled water. Dilute 5 ml. of this solution to 1 liter with redistilled water.

1 ml. = 0.5 micrograms Co

References

- Nichol, W.E. Microdetermination of cobalt colorimetrically as the 1-nitroso-2-naphthol complex. Can. J. Chem. 31: 145-149. 1953.
- Evans, B.S. The determination of cobalt. Analyst 62: 363-377. 1937.

REDUCING SUGARS

Method No. 1

Principle

Volumetric method. In slightly alkaline boiling solution reducing sugars are quantitatively oxidized by potassium ferricyanide. The excess ferricyanide is then allowed to react at room temperature with a solution of potassium iodide. Iodine is released quantitatively and is titrated immediately against standard sodium thiosulphate, with starch as an indicator.

Reagents

1. Potassium ferricyanide -- $K_3Fe(CN)_6$, 8.25 g.

Sodium carbonate-- Na_2CO_3 , 10.60 g.

Dissolve both salts in water and make to a volume of 1 liter.

Solution must be stored in a brown bottle.

2. Potassium iodide-- KI, 12.5 g.

Zinc sulphate-- $ZnSO_4 \cdot 7H_2O$, 25.0 g.

Sodium chloride-- NaCl, 125.0 g.

Dissolve the three salts in water and make to a volume of 500 ml.

Solution must be stored in a brown bottle and filtered before use if traces of iodine are apparent.

3. Acetic acid-- $HC_2H_3O_2$, 5% (v/v)

4. Starch indicator

Weigh 1 g. starch (for iodimetry) into a small beaker. Add 20 ml. water and mix. Pour suspension rapidly into 60 ml. boiling water. Continue boiling for 2 minutes. Add 20 g. sodium chloride. Cool, make to a volume of 100 ml. Solution will keep indefinitely.

5. Potassium iodate-- KIO_3 , 0.080% solution

6. Potassium iodide-- KI, 2% solution

7. Sodium thiosulphate-- $Na_2S_2O_3 \cdot 5H_2O$, 0.333% solution

This solution should be used in a 10-ml. burette graduated in 0.02-ml divisions.

Standardization: Measure 5 ml. potassium iodate solution (Reagent 5) into a large boiling tube. Add 5 ml. potassium iodide (Reagent 6) and 3 ml. acetic acid (Reagent 3). Titrate with sodium thiosulphate, using starch indicator.

$$\text{Normality} = \frac{\text{g. } KIO_3 \text{ per liter} \times \text{ml. } KIO_3 \text{ solution used}}{35.67 \times \text{ml. thiosulphate solution required}}$$

8. Glucose-- $C_6H_{12}O_6$

Procedure

Measure 5 ml. reducing sugar solution or extract into a large boiling tube. Add 5 ml. Reagent 1. Cover the tube loosely, preferably with a glass bulb, and place in boiling water for 15 minutes. Cool immediately in a cold water bath for 3 minutes. Add 5 ml. Reagent 2, 3 ml. Reagent 3 and titrate with standard sodium thiosulphate (Reagent 7) using a starch indicator (Reagent 4) which should be added near the end of the titration when the iodine color is almost completely discharged. A blank determination must be made simultaneously using 5 ml. water in place of the sugar solution. The difference between the titer for the water blank and that for the sugar solution gives the thiosulphate equivalent of the ferricyanide reduced by the sugar. For convenience in calculation and for use with the standard curve all titration values should be calculated to terms of 0.01 N sodium thiosulphate. In practice a water blank need be determined only once or twice a day.

Calculation

0.01 N thiosulphate equivalent = $(WB - R) \times N / 0.01$

WB = water blank titer

R = sugar solution titer

N = normality of thiosulphate solution

Standard

Dry a sample of glucose (Reagent 8) over phosphorus pentoxide at 78°C. in a vacuum drying apparatus. Dissolve 2.000 g. glucose in water and make to a volume of 1 liter. Dilute 20 ml. of this solution to 50 ml.

5 ml. = 4 mg. glucose

To prepare a standard curve use aliquots of this solution from 0.5 to 5.0 ml. making each aliquot to a total volume of 5 ml. with water.

References

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REDUCING SUGARS

Method No. 2

Principle

Volumetric method. In slightly alkaline boiling solution, reducing sugars are quantitatively oxidized by potassium ferricyanide. The ferrocyanide formed in the reaction is titrated against ceric sulphate in acid solution with Setopaline C as the indicator. The ferrocyanide is reconverted to ferricyanide giving a measure of the reducing sugars originally present.

Reagents

1. Potassium ferricyanide-- $K_3Fe(CN)_6$, 8.25 g.
Sodium carbonate-- Na_2CO_3 , 10.60 g.
Dissolve both salts in water and make to a volume of 1 liter. Store in a brown bottle.
2. Setopaline C
Dissolve 0.1 g. in 100 ml. water. On standing, a blue-green precipitate forms. Shake well before using.
3. Ceric sulphate-- $Ce(SO_4)_2$
Stock solution, 0.3 N: To 300 ml. water in a large beaker add 30 ml. H_2SO_4 and 75 g. ceric sulphate. Heat on a steam bath until all the salt is dissolved. Add more water if necessary. Filter, cool, and make to a volume of 500 ml.
Standard solution, 0.01 N: To 50 ml. water add 50 ml. H_2SO_4 and 65 ml. of ceric sulphate stock solution. Make to a volume of 2 liters with water. Standardize using pure glucose.
4. Sulphuric acid-- H_2SO_4 , 14.2 % (v/v)
Add acid to water slowly and with stirring. Cool and make up to volume.

Procedure

Measure 5 ml. reducing sugar solution or extract into a large boiling tube. Add 5 ml. Reagent 1. Cover the tube loosely, preferably with a glass bulb and place in boiling water for 15 minutes. Cool immediately in a cold water bath for 3 minutes. Add 5 ml. Reagent 4, 8 drops Reagent 2 and titrate with standard ceric sulphate solution (Reagent 3). A blank determination should be made on the reagents and the appropriate correction applied.

Standard

Dry a sample of glucose over phosphorus pentoxide at $78^\circ C$. in a vacuum drying apparatus. Dissolve 2.000 g. glucose in water and make to a volume of 1 liter. Dilute 20 ml. of this solution to 50 ml.

5 ml. = 4 mg. glucose

To prepare a standard curve use aliquots of this solution from 0.5 to 5.0 ml. making each aliquot to a total volume of 5 ml. with water.

References

Hassid, W. Z. Determination of sugars in plants by oxidation with ferricyanide and ceric sulphate titration. Ind. & Eng. Chem. Anal. Ed. 9: 228-229. 1937.

SUCROSE

Principle

Volumetric method. In the presence of hydrochloric acid in hot solution sucrose is quantitatively hydrolyzed to glucose and fructose. These reducing sugars can then be measured by any of several methods for determining reducing value.

Reagents

1. Hydrochloric acid-- HCl, S. G. 1.1 : 54% (v/v)
2. Sodium hydroxide-- NaOH, 25% solution
3. Sucrose-- $C_{12}H_{22}O_{11}$

Procedure

Measure 10 ml. of sugar extract into a large boiling tube. Add 10 ml. dilute HCl (Reagent 1) and heat at 70°C. for 9 minutes. Cool immediately in a cold water bath and allow to stand for 30 minutes. Neutralize with sodium hydroxide (Reagent 2) using litmus paper as an external indicator. The tube must be set in a cold water bath during the neutralization. Transfer solution to a 50-ml. volumetric flask, make to volume with water and determine the reducing value of the extract by one of the methods outlined on the previous pages.

Standard

Using a sample of sucrose, (Reagent 3) prepare a standard curve for invert sugar by the same procedure outlined for glucose in the reducing sugar method. The sucrose is first inverted by the procedure outlined above. To calculate sucrose from the invert sugar curve use a factor of 0.95.

STARCH

Method No. 1

Principle

Volumetric method. In the presence of the enzyme preparation takadiastase at a controlled pH, starch is quantitatively hydrolyzed to glucose which can be measured by any of several methods for determining reducing value.

Reagents

1. Takadiastase, 1% solution

Dissolve 1 g. takadiastase in 60 ml. water. Place solution in a dializer bag, seal the bag and allow it to stand in running water for 18 hours. Remove solution from the bag and make to a volume of 100 ml.

2. Acetic acid-- $\text{HC}_2\text{H}_3\text{O}_2$, 5% (v/v) solution
3. Toluene-- $\text{C}_6\text{H}_5\text{CH}_3$
4. Neutral lead acetate-- $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$, saturated solution
5. Sodium carbonate-- Na_2CO_3

Procedure

Dry the residue from the alcohol extraction of plant tissue described on page 6. Weigh the dried residue and, depending upon the expected starch content, weigh a portion or the whole residue into an Erlenmeyer flask. Add 90 ml. water and heat in a boiling water bath for 30 minutes. Cool to room temperature, add 10 ml. purified takadiastase (Reagent 1) and two drops acetic acid (Reagent 2). Mix well by shaking, add enough toluene to cover the surface of the solution and incubate at a temperature of 38°C . for 24 hours. Heat the mixture to boiling to inactivate the enzyme and to eliminate the toluene, Cool, filter, and wash. Clear the solution with neutral lead acetate and sodium carbonate by the procedure described for the preparation of extract of soluble sugars on page 6 and make the final solution to a volume of 100 ml. Determine the reducing value of the extract by one of the standard methods. A blank determination should be run on the takadiastase reagent. To calculate the amount of starch in the extract use the glucose standard curve described previously and a factor of 0.90.

References

Widdowson, E. M. Physiology of apples. 13. Starch and hemicellulose contents of developing apples. Ann. Bot. 46: 597-631. 1932.

STARCH

Method No. 2

Principle

Polarimetric method. Starch is dissolved in hot calcium chloride under uniform conditions. Soluble proteins are removed by precipitation and starch is determined by measurement of its optical rotation in a polarimeter or saccharimeter.

Reagents

1. Calcium chloride solution

Dissolve crystalline calcium chloride, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ or $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, in water and adjust the density of the solution to 1.30 at 20°C. using a hydrometer. Filter with suction and adjust the pH of the solution to 2.1 with acetic acid.

2. Uranyl acetate solution-- $\text{UO}_2(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$

Dissolve 5 g. in 100 ml. of Reagent 1.

3. Capryl alcohol

4. Carrez's solution

(a) 21.9 g zinc acetate, $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ + 3.0 ml. glacial acetic acid made up to 100 ml.

(b) potassium ferrocyanide, $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$, 10.6% solution

Procedure

Weigh 2.463 g. dried ground sample into a 400-ml. beaker. Add 10 ml. water and prepare a smooth suspension with the aid of a glass rod. Add 60 ml. calcium chloride solution (Reagent 1), stirring constantly. A few drops of acetic acid may be added at this point to avoid subsequent filtering difficulties if it is found that the solution after starch extraction has a pH greater than 3.0. Mark the level of the contents in the beaker. Bring solution to boiling within five minutes, frequently stirring. Allow boiling to proceed briskly for 17 minutes. During the heating period maintain the volume of liquid by small additions of water to ensure uniformity in salt concentration and extraction temperature. As an alternative procedure to the boiling just described, the solution after addition of the calcium chloride reagent may be autoclaved for 10 minutes at a pressure of 15 lb. per sq. in. A few drops of capryl alcohol (Reagent 3) may be added if frothing occurs. Cool immediately to room temperature and add 10 ml. uranyl acetate solution (Reagent 2) with stirring or 2 ml. each of the two Carrez's solutions (a) and (b) (Reagent 4). Transfer solution to a 100-ml. volumetric flask and make to volume with calcium chloride solution (Reagent 1). Filter, discard the first 20 ml. and collect the filtrate for polarization at 20°C. on a polarimeter or saccharimeter.

Calculation

$$\frac{A \times 100 \times 100}{203 \times S \times L} = \text{per cent starch in sample}$$

A = angular rotation

S = weight of sample in grams

L = length of tube in decimeters

1° Ventzke scale on saccharimeter = 0.34657° angular rotation D

References

- Clendenning, K.A. Polarimetric determinations of starch in cereal products.
Can. J. Research 23B: 113-130. 1945.
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21: 267-275. 1944.
- Hopkins, C.Y. Improvements in the determination of starch in corn and wheat.
Can. J. Research 11: 751-758. 1934.
- Fraser, J.R., M. Brandon-Bravo, and D.C. Holmes. The proximate analysis
of wheat flour carbohydrates. J. Sci. Food Agric. 9: 577-589. 1956.

STARCH

Method No. 3

Principle

Colorimetric method. Starch is dissolved in perchloric acid at a controlled concentration. Upon the addition of iodine a blue starch-iodide complex is formed which is measured colorimetrically.

Reagents

1. Perchloric acid-- HClO_4 , 72 %
2. Phenolphthalein indicator-- 1 % solution in 95 % ethanol
3. Sodium hydroxide-- NaOH , 2N solution (8 %)
4. Acetic acid-- CH_3COOH , 2N solution (11.43 % v/v)
5. Potassium iodide-- KI , 10 % solution
6. Potassium iodate-- KIO_3 , 0.0125 N (0.0446 %)
7. Potato starch

Procedure

Weigh 50-100 g. fresh tissue into a Waring blender jar. Add an equal weight of water. Blend for 5 minutes. Scrape down the walls and lid of the blender and mix the sample thoroughly. Rapidly weigh 2 g. of slurry into a 30-ml. beaker, keeping the mixture well stirred while measuring. To the aliquot in the beaker add 2 ml. water and 2.7 ml. perchloric acid (Reagent 1) by drops stirring vigorously. Allow to stand 10 minutes with occasional stirring. Transfer the mixture quantitatively with water to a 50-ml. volumetric flask and make up to volume. Allow mixture to settle, then measure an aliquot, depending upon starch content, into another 50-ml. volumetric flask and add about 10 ml. of water. Add 1 drop phenolphthalein indicator (Reagent 2). Add sodium hydroxide (Reagent 3) until a pink color appears. Add acetic acid (Reagent 4) until the pink color disappears, then add an additional 2.5 ml. Add 0.5 ml. potassium iodide solution (Reagent 5) and 5.0 ml. potassium iodate solution (Reagent 6). Shake the flask, allow it to stand for 5 minutes, then make up to volume. Read absorbance in a photoelectric colorimeter using a No. 66 red filter. Set the instrument scale at zero with water.

If dried tissue is used, the procedure may be started by weighing an appropriate sample into a 30-ml. beaker, and adding 4 ml. water and perchloric acid as described.

Standard

Weigh 0.050 g. pure potato starch into a 30-ml. beaker. Add 4 ml. water and 2.7 ml. perchloric acid by drops with vigorous stirring. Allow to stand 10 minutes. Transfer quantitatively with water to a 50-ml. volumetric flask and make to volume.

1 ml. = 1 mg. starch

To prepare a standard curve use aliquots of this solution from 0.1 ml. to 3.0 ml. following the procedure outlined above.

Since starches from different species of plants vary widely in their amylose content, it is preferable to employ as a standard a sample of pure starch from the species of plant being analyzed. If this cannot be done, use a recorded or experimental value for amylose content and apply an appropriate correction using a value of 26.4% amylose for potato starch.

References

Nielsen, J. P. Rapid determination of starch. Ind. & Eng. Chem. Anal. Ed. 15: 176-179. 1943.

MISCELLANEOUS METHODS

In the foregoing pages no procedures are outlined for a few constituents that are frequently determined in this laboratory and for certain other constituents that are occasionally determined on samples of plant tissue for special reasons. Following is a list of these constituents.

- Ether Extract
- Crude Fiber
- Lignin
- Silica
- Barium
- Arsenic
- Selenium
- Nickel
- Iodine
- Fluorine

Procedures for the analysis of most of these constituents may be found in the Official Methods of Analysis of the A. O. A. C. , to which reference has previously been made.

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